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13. ABSTRACT *(Maximum 200 words)*

This project evaluated the toxicity and safety of several drugs under development. During the contract, tasks conducted included: mutagenicity tests; developmental toxicity studies in rats and rabbits; acute toxicity studies in rats and mice; two, four and thirteen week toxicity studies in rats and dogs; a six month toxicity study in rats; fertility/early embryonic development studies in rats; a perinatal toxicity study in rats; and a one year toxicity study in dogs. Drugs studied included: WR242511 Tartrate, WR238605 Succinate, Halofantrine HCl, Pyridostigmine Bromide, WR6026 Dihydrochloride, HI-6 Dichloride, WR269410, Atropine, WR279396, and Ampicillin.

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FOREWORD

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__X__ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

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____ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Barry S. Levine 4/15/98

PI - Signature Date

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1A. SUBMITTED DRAFT REPORTS (11/15/96 - 04/15/98)

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219	UIC-9	One Year Oral Toxicity Study of WR238605 Succinate in Dogs	12/19/97

2. SUMMARIES AND CONCLUSIONS OF STUDIES

Study No. 090 Amended (Task Order UIC-2)

This study evaluated the toxicity of WR6026 in dogs following thirteen weeks of daily oral (capsule) administration. A thirteen week recovery period was included for control, mid and high dose groups. Dose levels studied were 0 (vehicle control), 0.2, 2.0 and 3.0 mg base/kg/day. The primary toxic effects of WR6026 included methemoglobinemia with clinical signs of cyanosis, mild hemolytic anemia, leukocytosis, transient thrombocytopenia and hepatotoxicity. These effects were seen at the high and mid dose levels, with sporadic mild cyanosis apparently secondary to marginal methemoglobinemia, and mild hepatocyte necrosis also noted in low dose animals. WR6026-induced toxicity was essentially reversible at the end of the three month recovery period. On the basis of the present investigation, a no observed adverse effect level was not obtained.

Study No. 091 (Task Order UIC-2)

This study evaluated the toxicity of WR6026 in rats following thirteen weeks of daily oral administration by gavage. A thirteen week recovery period was included for control, mid and high dose groups. Dose levels studied were 0 (vehicle control), 3, 6 and 12 mg base/kg/day. The primary toxic effects of WR6026 included anemia, leukocytosis, transient thrombocytopenia, methemoglobinemia clinically manifested by cyanosis, and mild hepatotoxicity. In general, females appeared to be more sensitive than males to WR6026 treatment. These effects were generally seen in the high and mid dose levels, except for elevated serum ALT and AST levels (suggestive of liver toxicity) only observed at the high dose. The apparent nephrotoxicity at the high dose was considered secondary to the anemic state. Transient changes at the low dose included thrombocytopenia and leukocytosis. Low dose animals also demonstrated marginal cyanosis in the absence of significant methemoglobinemia. As such, a no effect level was not obtained in this study. Essentially complete recovery from the toxic effects of WR6026 occurred by the end of the three month recovery period.

Study No. 094 (Task Order UIC-2)

This study evaluated the dispersement *in vitro* of WR6026 and its clinical tolerance in dogs following daily oral administration for three days. The test article (40 mg base) in gelatin capsules readily dissolved in an artificial dog stomach medium and there was no evidence of intolerance after dosing dogs with undiluted WR6026 in gelatin capsules. Based on the results of these studies, the use of undiluted test article in gelatin capsules was recommended as the dosage formulation for the upcoming 13 week study (Study No. 090).

Task Order UIC-3

The purpose of this project was to examine the mutagenicity of HI-6 Dichloride. The following five *in vitro* studies were conducted at Microbiological Associates, Inc. via a subcontract mechanism.

Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test)

L5178Y TK+/- Mouse Lymphoma Mutagenesis Assay

CHO/HGPRT Mutation Assay

Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells

Chromosomal Aberrations in Human Blood Lymphocytes

HI-6 Dichloride was shown to be positive in the two chromosomal aberrations tests while being negative in the remaining three assays which tested for point mutations. Accordingly, an *in vivo* cytogenetics test in rats was conducted as a confirmatory study. In that study, two samples of HI-6 Dichloride were tested; one which was synthesized using bischloromethylether (BCME), and one which was synthesized by a non-BCME route. Both samples were negative in the *in vivo* assay. It is therefore concluded that the overall mutagenic hazards associated with exposure to HI-6 Dichloride are negligible.

Task Order UIC-4

The purpose of this project was to examine the oral toxicity of two topical skin protectants in rats following two weeks of gavage administration. Protocols were submitted, however, the studies were never conducted as directed by the Sponsor.

Study No. 097 (Task Order UIC-5)

This study evaluated the toxicity of WR238605 in dogs following thirteen weeks of daily oral (gavage) administration. A thirteen week recovery period was included for all groups. Dose levels studied were 0 (vehicle control), 0.1, 2.0 and 6.0 mg base/kg/day. The primary toxic effects of WR238605 were seen in the lungs, RBCs, and liver. Drug treatment was associated with hemolytic anemia which was supported by reticulocytosis, bone marrow hypercellularity, decrease in bone marrow M/E ratio, splenomegaly, extramedullary hematopoiesis, and hemosiderosis in the liver and spleen. Mild hepatotoxicity as evidenced by hepatocyte necrosis (high dose males) was supported by altered clinical chemistry values. Apparent congested retinal vessels was seen in one high dose female, which was no longer evident by the end of the recovery period. Possible generalized or secondary toxic effects related to the stress produced by the anemic and/or methemoglobinemic state included decrease in weight gain; neutrophilic and monocytic leukocytosis; and depletion of thymic lymphocytes. Methemoglobinemia was manifested by clinical signs of cyanosis (blue gums, tongue, and sclera). Lung lesions induced by WR238605 included alveolar proteinosis and subacute inflammation. The above described toxic effects were generally seen at the high and mid dose levels, although mild cyanosis and hemosiderosis secondary to hemolytic anemia, subacute inflammation in the lungs and liver, and bone marrow hypercellularity were also seen to a limited extent in low dose animals. WR238605 toxicity was essentially reversible, except for the lung lesions and the microscopic changes secondary to the observed hemolytic anemia, e.g. hemosiderosis.

Study No. 098 (Task Order UIC-5)

This study evaluated the toxicity of WR238605 in rats following thirteen weeks of daily oral (gavage) administration. A thirteen week recovery period was included for all groups. Dose levels studied were 0 (vehicle control), 0.5, 6 and 18 mg base/kg/day. The primary toxic effects were seen in the RBCs, lungs and liver. Significant methemoglobin production was observed in mid and high dose animals, but was reversible. Microscopic lesions in the spleen, kidney, and bone marrow were secondary to mild hemolytic anemia. Toxicity again was limited to the two highest dose levels. Decreased food consumption, decreased body weight gains, methemoglobin production and mild anemia were observed at the mid and high dose levels, but were readily reversible after treatment cessation. Increases in serum ALT, AST, and/or LDH and decreased A/G ratios in high dose animals and possible mid dose males suggested mild hepatotoxicity, however, histopathologic lesions were not seen. Leukocytosis possible secondary to stress and consisting of increased numbers of lymphocytes, mature neutrophils, and/or monocytes were seen in the treatment period at the two highest dose levels and was reversible after cessation of treatment. Because the aforementioned toxic responses were limited to mid and high dose animals, a no-adverse effect level of WR238605 was assessed to be 0.5 mg base/kg/day.

Study No. 139 (Task Order UIC-6)

This dose range-finding study evaluated the toxicity of microencapsulated ampicillin anhydrate following a single exposure in New Zealand White rabbits when applied into a surgically, aseptically-produced, deep muscle wound. The results were to be used to select dose levels for a main toxicity study. The study included four groups of animals which were either sham-treated (surgery only), treated with 500 mg of microcapsule placebo (MP), treated with 250 mg of microencapsulated ampicillin anhydrate (MAA) or treated with 500 mg of MAA (core weight was 28.2 wt % ampicillin). No apparent test article-related changes were observed in the study. Myocardial changes seen in one high dose MAA-treated animal were probably spurious. Other microscopic changes and alterations in body weights and clinical pathology parameters were considered to be related to the surgical procedure or the implantation of a foreign material into the thigh wound, and not related to ampicillin treatment. Plasma ampicillin levels were detected in seven of eight MAA-treated rabbits 1 hour after treatment and generally declined thereafter at the later time points tested. Based upon the aforementioned findings, the no observed effect level (NOEL) was probably 500 mg microencapsulated ampicillin anhydrate, the high dose of MAA in the present study. This dose level is therefore suitable as the high dose in the subsequent main toxicity study in rabbits.

Study No. 140 (Task Order UIC-6)

This study to evaluate the toxicity of microencapsulated ampicillin anhydrate following a single exposure in New Zealand White rabbits when applied into a surgically, aseptically-produced, deep muscle wound was cancelled by the Sponsor.

Study No. 104 (Task Order UIC-7)

This study examined the acute oral and intraperitoneal toxicity of WR242511 Tartrate and

WR269410 in rats. The dose levels were selected on the basis of range-finding tests. After dosing, the animals were weighed weekly, observed daily for 14 days, and the survivors were necropsied on Day 14. Nonsurvivors were also necropsied. The acute oral LD50 of WR242511 Tartrate in male rats, administered in 1% Methylcellulose/0.4% Tween 80 by gavage, was approximately eight-fold lower than in female rats (males; 16.3 mg base/kg and females; 135 mg base/kg). The LD50 values were not significantly different between the sexes (males; 23 mg base/kg and females; 30 mg base/kg). Thus, the LD50 of WR242511 Tartrate was unaffected by sex when administered by intraperitoneal injection, but affected by sex when administered orally.

The acute oral LD50 of WR269410 in male rats, administered in 1% Methylcellulose/0.4% Tween 80 by gavage, was approximately three-fold greater than in female rats (males; 420 mg/kg and females; 147 mg/kg). Due to a physical inability to intraperitoneally administer WR269410 dosage formulations in 0.1% Methylcellulose/0.4% Tween 80 at high enough concentrations to produce lethality, WR269410 was administered intraperitoneally as a solution in polyethylene glycol 200 (PEG 200). The calculated LD50 value of intraperitoneally administered WR269410 in males was 155 mg/kg. An LD50 value in females was estimated to be approximately 70 - 80 mg/kg.

The results of this study suggest that WR242511 Tartrate is more acutely toxic than WR269410 when administered either orally or intraperitoneally. Based on the oral LD50 data and after consultation with the Sponsor, the following dose levels were suggested to be used in the two week oral dose range-finding studies in rats of WR242511 Tartrate; 0, 0.5, 2.0, and 6.2 mg base/kg/day, and of WR269410; 0, 2.0, 6.0, 18.0 mg/kg/day.

Study No. 105 (Task Order UIC-7)

This study examined the acute oral and intraperitoneal toxicity of WR242511 Tartrate and WR269410 in mice. The dose levels were selected on the basis of range-finding tests. After dosing, the animals were weighed weekly, observed for 14 days, and the survivors were necropsied on Day 14. Nonsurvivors were also necropsied. The acute oral LD50s of WR242511 Tartrate, administered in 1% methylcellulose/0.4% Tween 80 by gavage, were not significantly different between sexes (males; 23.0 mg base/kg and females; 22.45 mg base/kg). The LD50 values obtained when WR242511 Tartrate was administered intraperitoneally in the same vehicle were identical for both sexes (21.82 mg base/kg). Thus, the LD50 of WR242511 Tartrate was unaffected by sex or route of administration.

Due to dosage formulation problems, WR269410 was administered as a solution in polyethylene glycol 200 (PEG 200). An oral LD50 could not be calculated because of apparent vehicle lethality at the dosing volumes necessary to administer lethal doses of WR269410. The oral LD50 of WR269410 is however estimated to exceed 1000 mg/kg, based on the additive contribution of the vehicle to the mortality observed. In the intraperitoneal acute toxicity test, WR269410 was administered in PEG 200 resulting in a LD50 value of 117.3 mg/kg in males and 190.04 mg/kg in females. Thus, WR269410 is several-fold less acutely toxic than WR242511 Tartrate administered by either route. This latter drug also demonstrated a significantly steeper dose-mortality curve.

Study No. 106 (Task Order UIC-7)

This study evaluated the toxicity of WR242511 Tartrate in rats following two weeks of daily oral administration by gavage. Dose levels studied were 0 (vehicle control), 0.5, 2.0 and 6.2 mg base/kg/day. The primary toxic effects of WR242511 Tartrate included anemia, hepatotoxicity and leukocytosis. Females were more sensitive than males to the anemic state whereas the reverse was true for hepatotoxicity. Anemia was seen in mid and high dose females, whereas hepatotoxicity was only observed in high dose males and may have been associated with the death of one high dose male on Day 13. Generalized leukocytosis occurred in the high dose animals and in mid dose females. Toxicity was not apparent in low dose animals. Significant methemoglobinemia was noted in mid and high animals, and possible at the low dose. As this is the desired pharmacologic effect of WR242511 Tartrate, its occurrence was not considered indicative of toxicity. The purpose of this study was to select dose levels for a three month toxicity study in rats. It is anticipated that significant toxicity would occur at the high dose, marginal or no toxicity would be observed at the mid dose, and no toxicity would occur at the low dose level. On this basis, the following three dose level ranges were suggested: 0.5, 1 - 1.5, and 2 - 4.5 mg base/kg/day.

Study No. 107 (Task Order UIC-7)

This study evaluated the toxicity of WR242511 Tartrate in rats following thirteen weeks of daily oral (gavage) administration. Dose levels studied were 0 (vehicle control), 0.5, 1.5 and 4.5 mg base/kg/day. The primary treatment-related toxic effects of WR242511 were seen in the liver, lungs and RBCs. Males appeared more sensitive than females to the hepatotoxic effects of WR242511 administration. Microscopic liver lesions (hepatocyte degeneration and necrosis), and elevations in serum ALT and/or SDH levels were observed in mid and high dose males. Increased triglyceride and cholesterol levels in high dose females, and increased cholesterol levels in high dose males also suggested potential hepatocellular toxicity. Increases in total bile acids and alkaline phosphatase levels suggested hepatobiliary changes in high dose animals. Pulmonary microscopic lesions (alveolar histiocytosis) were observed in all WR242511-treated groups. These dose-related effects (hepatocyte degeneration and necrosis, and alveolar histiocytosis) probably contributed to the early deaths of seven out of ten high dose males. Treatment-related mild anemia was observed in mid dose and high dose animals. Hemosiderosis in the spleen of high dose females was probably secondary to mild hemolytic anemia. Significant methemoglobin production was also observed in mid and high dose animals. The lesser methemoglobinemic response seen in high dose males compared to high dose females may have been secondary to the greater hepatotoxic effect in males, resulting in a reduction in the production of a direct methemoglobin-forming metabolite. Thymic lymphocyte depletion in high dose males was apparently secondary to stress produced by test article administration, but possibly could also be a direct treatment-related effect. Mild leukocytosis possibly secondary to stress and consisting of increased number of lymphocytes, neutrophils, monocytes, and/or eosinophils was seen in high dose animals and mid dose males. Thrombocytopenia was observed in all WR242511-treated groups. Because alveolar histiocytosis, thrombocytopenia, and hematology changes were seen at the low dose level, a no-adverse effect level of WR242511 could not be determined.

Study No. 112 (Task Order UIC-7)

This study evaluated the toxicity of WR269410 in rats following two weeks of daily oral administration by gavage. Dose levels studied were 0 (vehicle control), 2.0, 6.0, and 18.0 mg/kg/day at study initiation. On Day 7, the mid dose level (6.0 mg/kg/day) was elevated above the high dose to 30 mg/kg/day for the second treatment week due to a lack of significant toxicity at the high dose during the first week of treatment. The primary toxic effect of WR269410 was hemolytic anemia, which was supported by macrocytosis, reticulocytosis, Heinz bodies, splenomegaly and extramedullary hematopoiesis. Females were more sensitive than males to the anemic state. Anemia was seen in males at the two higher doses, but was apparent in all female treatment groups. Methemoglobinemia, the expected pharmacologic effect, was also observed at all three dose levels. As this is the desired pharmacologic effect of WR269410, its occurrence was not considered indicative of toxicity. Cardiomegaly, possible secondary to the methemoglobinemic and anemic state, was seen only in females at 6.0/30.0 mg/kg/day. The purpose of this study was to select dose levels for a three month toxicity study in rats. It is anticipated that significant toxicity would occur at the high dose, marginal or no toxicity would be observed at the mid dose, and no toxicity would occur at the low dose level. On this basis, the following dose levels are suggested: 0, 1, 2.5 and 6 mg/kg/day. After consultation with the Sponsor, the following dose levels were chosen for the three month toxicity study in rats: 0, 1, 3, and 10 mg/kg/day.

Study No. 113 (Task Order UIC-7)

This study evaluated the toxicity of WR269410 in rats following thirteen weeks of daily oral (gavage) administration. Dose levels studied were 0 (vehicle control), 1, 3 and 10 mg/kg/day. The primary toxic affect of WR269410 treatment was hemolytic anemia. The diagnosis of anemia was supported by anisocytosis, polychromasia, macrocytosis, reticulocytosis, Heinz bodies, Howell-Jolly bodies, splenomegaly, splenic extramedullary hematopoiesis, and hemosiderosis in the liver and spleen. Dose-related anemia and methemoglobinemia were observed in all WR269410-treated groups. Slight leukocytosis possibly secondary to stress and consisting of increased numbers of mature neutrophils was seen in the high dose males, but not females, or other dose groups. Because subtle effects of the aforementioned anemia including secondary histologic changes (splenic hemosiderosis and extramedullary hematopoiesis) were observed in low dose animals, a no-observed effect level of WR269410 could not be determined in the present study.

Study No. 133 (Task Order UIC-7)

This study evaluated the toxicity of WR242511 tartrate in dogs following four weeks of daily administration by gelatin capsule. The primary toxic effects of WR242511 tartrate were seen in the RBCs, liver and the lung. Anemia and methemoglobinemia were observed in all the dose levels tested. Compensatory changes to the anemic state included macrocytosis, reticulocytosis and increased nucleated RBCs. Decreases in body weight and food consumption were seen at the high dose level and possibly the mid dose level. Microscopic lesions were seen in the liver and the lung at all dose levels tested. Hepatocellular swelling was supported by decreases in the A/G ratio and increases in haptoglobin levels in mid and/or high dose animals. Pulmonary lesions

(alveolar proteinic exudates, macrophage infiltration and acute alveolar inflammation) and mild to moderate thrombocytopenia were seen at all dose levels. Leukocytosis consisting of increased mature neutrophils, possibly secondary to stress, was seen in high animals and possibly in the mid dose female. On the basis of the findings from this study and after consultation with the Sponsor, the following three dose levels have been selected for use in the four week oral toxicity study: 0.1, 0.3 and 1.0 mg base/kg/day.

Study No. 134 (Task Order UIC-7)

This study evaluated the toxicity of WR242511 tartrate in dogs following four weeks of daily administration by gelatin capsule. Dose levels studied were 0, 0.1, 0.3 and 1.0 mg base/kg/day. The primary toxic effects of WR242511 were seen in the RBCs, lungs and platelets. Although subtle, hemolytic anemia was supported by reticulocytosis, secondary splenic extramedullary hematopoiesis and bone marrow hyperplasia in high dose animals. A slight, statistically insignificant decrease in body weight (-0.6 kg) was also seen in high dose males and females. Methemoglobinemia, the desired pharmacologic effect, accompanied by clinical signs of cyanosis (blue gums, tongue and sclera), and mild to moderate thrombocytopenia were observed in mid and high dose animals. WR242511 treatment induced interstitial pulmonary inflammation in seven out of eight high dose animals. Minimal, but significant increases in serum AST, globulin, and triglyceride levels in high dose males and decreases in albumin levels and A/G ratio in both high dose males and females, not accompanied by corresponding histopathologic changes in the liver, suggests that WR242511 is marginally hepatotoxic. Additionally, increased serum haptoglobin levels, indicative of an acute phase reaction, were observed in mid dose males and high dose animals. Because the aforementioned toxic responses were limited to the mid and high dose levels, the no-observed effect level (NOEL) of WR242511 tartrate was 0.1 mg base/kg/day.

Study No. 135 (Task Order UIC-7)

This study evaluated the toxicity of WR269410 in dogs following four weeks of daily administration by gelatin capsule. Because neither frank toxicity nor biologically significant increases in methemoglobin production were seen at the initial dose levels studied (1.0, 2.5 and 6.0 mg/kg/day), the dose levels were increased in animals receiving 6.0 mg/kg/day to 12.0 mg/kg/day (starting Day 8) and for the animals receiving 2.5 mg/kg/day to 24.0 mg/kg/day (starting Day 15). The primary toxic effect of WR269410 was hemolytic anemia, supported by reticulocytosis, nucleated RBCs, Heinz bodies and secondary splenic extramedullary hematopoiesis. Dose-related anemia, methemoglobinemia and microscopic changes in the liver (swollen hepatocytes and cholestasis) were observed at the 6.0/12.0 and 2.5/24.0 mg/kg/day dose levels. On the basis of these findings and after consultation with the Sponsor, the following dose levels have been selected for use in the four week oral toxicity study: 2, 7 and 24 mg/kg/day.

Study No. 136 (Task Order UIC-7)

This study evaluated the toxicity of WR269410 in dogs following four weeks of daily administration by gelatin capsule. Dose levels studied were 0, 2, 7 and 24 mg/kg/day. The primary toxic effect of WR269410 was hemolytic anemia. The diagnosis of anemia was supported by anisocytosis, polychromasia, macrocytosis, reticulocytosis, nucleated RBCs, Heinz bodies,

splenomegaly, secondary splenic extramedullary hematopoiesis and bone marrow hyperplasia in mid and high dose animals. Additionally, decreased haptoglobin levels observed in mid and high dose animals further supported hemolysis as the origin of the anemia. A slight, statistically insignificant decrease in body weight (-0.7 kg) was also seen in high dose females but not males. Methemoglobinemia, the desired pharmacologic effect, accompanied by clinical signs of cyanosis (blue gums, tongue and sclera and white/grey gums and tongue), was primarily limited in mid and high dose animals. Increases in serum total bilirubin levels in mid and high dose animals, not accompanied by corresponding histopathologic changes, suggests that WR269410 induced marginal hepatobiliary changes. Thrombocytosis was observed in mid dose males and in high dose animals. Because the aforementioned toxic responses were limited to the mid and high dose levels, the no-observed effect level (NOEL) of WR269410 was determined to be 2.0 mg/kg/day.

Study No. 137 (Task Order UIC-7)

This dose range-finding study evaluated the developmental toxicity of WR242511 tartrate in time-mated New Zealand White (Pasteurella Free) female rabbits. Doses were 0, 0.5, 1, 2.5, 6 and 14 mg base/kg/day administered by gavage during gestation days (GD) 6 - 18 (GD0 = day of observed mating). The doses were based on a preliminary dose range-finding study of WR242511 in non-pregnant rabbits and a dose range-finding developmental toxicity study in rats. All animals in the 6 and 14 mg base/kg/day doses were dead by GD12. Changes in their reproductive indices (e.g. % total loss, % preimplantation loss) were a reflection of early maternal mortality. In the 2.5 mg base/kg/day dose, marginal maternal toxicity was indicated by biologically, but not statistically, significant decreases in food consumption at GD15 and GD18 (i.e. only towards the end of dosing), accompanied by a marginal loss of weight in one of these pregnant rabbits. The 2.5 mg base/kg/day dose was therefore considered at or near the low observable adverse effect level (LOAEL) for maternal toxicity.

Fetal toxicity was apparent in the 2.5 mg base/kg/day dose, and included one non-viable fetus. Biologically significant decreases in fetal body weights were also observed in this dose and in 1 mg base/kg/day female fetuses. This decrease was also statistically significant in the female fetuses at 2.5 mg base/kg/day. No other test article-related differences were observed in any other fetal parameters across groups. The 1 mg base/kg/day dose was considered at or near the low observable adverse effect level (LOAEL) in the fetuses. Accordingly, the following doses are recommended for the definitive developmental toxicity (Segment II) study in rabbits: 0, 0.5, 1.3 and 3.5 mg base/kg/day.

Study No. 138 (Task Order UIC-7)

This study evaluated the embryo/fetal toxicity and the teratogenic potential of WR242511 tartrate in time-mated New Zealand White (Pasteurella Free) female rabbits. Doses were 0, 0.5, 1.3, and 3.5 mg base/kg/day administered by gavage during gestation days (GD) 6 - 18 (GD0 = day of observed mating). In addition, a positive control group was administered retinol palmitate, 300 mg/kg/day, on GD9 and 10 by gavage. One female in the high dose prematurely delivered on GD29 and one female in the mid dose aborted on GD27. No other maternal toxic manifestations were observed in any WR242511 dose level. In addition, fetal toxicity was not apparent. In the positive control group, one female aborted on GD22. Other manifestations of toxicity in this

group were a marginal decrease in weight gain during dosing; significant decreases in uterine weight and viable fetuses; and significant increases in post-implantation loss, early resorptions and fetuses with external, visceral and skeletal malformations.

With the exception of one abortion and one premature delivery in test article-treated animals, toxicity was not apparent in either the does or their fetuses. Based on the results of this study, the highest dose tested (3.5 mg base/kg/day) was considered at or near the no observed effect level for both maternal and fetal toxicity in rabbits. Since 6 mg base/kg/day in a previously conducted dose range-finding study was lethal to 5/5 animals, it is believed that a dose in excess of 3.5 mg base/kg/day in the present investigation would have resulted in excessive mortality.

Study No. 141 (Task Order UIC-7)

This rabbit dose range-finding test to select doses to study the developmental toxicity of WR269410 in rabbits was cancelled by the Sponsor.

Study No. 142 (Task Order UIC-7)

This study to evaluate the developmental toxicity of WR269410 in rabbits was cancelled by the Sponsor.

Study No. 143 (Task Order UIC-7)

This dose range-finding study evaluated the developmental toxicity of WR242511 tartrate in time-mated CD® female rats. Doses were 0, 0.5, 1, 2, 4 and 8 mg base/kg/day administered by gavage during gestation days (GD) 6 - 15 (GD0 = day of vaginal plug). Maternal toxicity was observed at the high dose as a significant decrease in total weight gain. In addition, significant decreases in mean daily food consumption were seen during the treatment period. Rough coat was also observed in three females during GD13-15. The 4 mg base/kg/day dose was considered near or at the maternal no observable effect level (NOEL).

Fetal toxicity was apparent at 8 mg base/kg/day as significant decreases in body weights were seen. At the 4 mg base/kg/day dose, a biologically significant decrease in fetal mean body weights was observed, but was only statistically significant in female fetuses. However, at 1 mg base/kg/day a statistically significant decrease was present in both sexes. No biological differences in any other fetal parameters were observed at the high dose or mid high dose groups vs. the control group. The absence of an effect on fetal body weights at 2 mg base/kg/day could not be explained. Fetal body weight changes at 8 mg base/kg/day were considered due to and/or associated with maternal toxicity. The 1 mg base/kg/day dose was considered at or near the low observable adverse effect level (LOAEL) for fetal toxicity. Accordingly the following doses are recommended for the definitive developmental toxicity (Segment II) study in rats: 0, 0.5, 2 and 8 mg base/kg/day.

Study No. 144 (Task Order UIC-7)

This study evaluated the developmental toxicity of WR242511 tartrate in time-mated CD® female

rats. Doses were 0, 0.5, 2, and 8 mg base/kg/day administered by gavage during gestation days (GD) 6 - 15 (GD0 = day of vaginal plug). In addition, a positive control group was administered retinol palmitate, 1000 mg/kg/day, on GD9 and 10 by gavage. Maternal toxicity was observed at the mid and the high doses. Statistically significant decreases in body weights during GD10 - 14 and GD7 - 20 were observed in the two groups, respectively. In addition, associated decreases in total weight gain were significant at the high dose. Significant decreases in food consumption during GD6 - 15 and GD6 - 20 were also present in the mid and high dose groups, respectively. While rough coat was first seen in high dose animals on GD8, the majority of these animals generally showed rough coat from GD11 throughout the remainder of the study. Furthermore, pale appearance and hunched posture were observed at the high dose. Rough coat was also observed in the mid and low dose levels but for limited periods and on sporadic occasions. Gross necropsy revealed dose-related increases in the number of animals with enlarged spleens among the mid and the high dose groups. Marginal decreases in gravid uterine weight was also observed at the high dose. Apart from complete resorption in two females at the high dose, no other maternally-related reproductive cesarean section parameters or reproductive indices were affected. Females in the positive control group showed statistically significant reductions in body weight on GD10 - 13 and decreases in food consumption during GD6 - 10. Sporadic incidences of rough coat were also seen on GD9 - 12.

Fetal toxicity was only observed at the high dose (i.e., 8 mg base/kg/day) and in the positive control group. A statistically significant decrease in fetal weights was the only drug-related effect at the high dose. In the positive control group, significant decreases in fetal body weight and increases in the number of fetuses with external malformations and with visceral anomalies were observed. Accordingly, in this study, 0.5 mg base/kg/day of WR242511 tartrate was considered the maternal no observable effect level (NOEL), while the 2 mg base/kg/day dose was considered the developmental NOEL in CD® rats.

Study No. 145 (Task Order UIC-7)

This dose range-finding test to select doses to study the developmental toxicity of WR269410 in rats was cancelled by the Sponsor.

Study No. 146 (Task Order UIC-7)

This study to evaluate the developmental toxicity of WR269410 in rats was cancelled by the Sponsor.

In Vitro Mutagenicity Testing of WR242511 Tartrate (Task Order UIC-7)

WR242511 Tartrate was tested for point mutations and chromosomal aberrations in three *in vitro* mutagenicity tests (Ames Test, Chromosome Aberration Test using Chinese Hamster Ovary cells and Mouse Lymphoma Assay). WR242511 Tartrate was shown to be negative in the three assays utilized.

In Vitro Mutagenicity Testing of WR269410 (Task Order UIC-7)

WR269410 was tested for point mutations and chromosomal aberrations in three *in vitro* mutagenicity tests. WR269410 was shown to be negative in the Ames Test and the Chromosome Aberration Test using Chinese Hamster Ovary cells, but induced point mutations in the Mouse Lymphoma Assay when a 9000 g rat liver preparation was included.

Study No. 129 (Task Order UIC-8)

This dose range-finding study evaluated the toxicity in dogs of co-administered HI-6 dichloride monohydrate (HI-6 dm) and atropine citrate (ATC) in the formulation used in Atropen autoinjector following one week of daily administration by intramuscular injection. The dose levels studied were 70 mg/kg/day of HI-6 dm co-administered with ATC dose levels of 0.10, 0.25, 0.50 or 1.0 mg base/kg/day. No animals died during the study. Treatment with the test article dosing solutions produced a painful stimulus as judged by escape behavior and vocalization. The noxious stimuli may account for the occasional emesis observed, possibly stress-induced, following treatment. Decreased activity was observed in the three highest ATC dose levels. The ATC high dose female was also observed to be lethargic. Because these clinical signs of toxicity were not seen after day 3, it appeared that tolerance developed to these CNS effects. Mydriasis, a pharmacologic (anti-muscarinic) action of atropine, was generally observed throughout the study at all dose levels. Slight decreases in body weights accompanied by no apparent decreases in food intake were observed in males. Only the high dose female had body weight loss, which was accompanied by decreased food intake. Based on these findings and the premise that tolerance develops to decreased activity, following consultation with the Sponsor, a dose level of 1.0 mg base/kg/day ATC was selected for use in a two week intramuscular toxicity study of co-administered HI-6 dm and ATC.

Study No. 130 (Task Order UIC-8)

This study evaluated the toxicity in dogs of co-administered HI-6 dichloride monohydrate (HI-6 dm) and atropine citrate (ATC), the formulation used in the Atropen autoinjector, following two weeks of daily administration by intramuscular injection. The dose levels of HI-6 dm and ATC were 0, 35 or 70 mg/kg/day and 0 or 1 mg base/kg/day, respectively. The control group received the vehicle (ATC placebo) which was the Atropen autoinjector formulation without atropine. The administration of the various dosing solutions produced significant pain as evidenced by vocalization and escape behavior and required the use of two technicians to forcefully restrain the dogs while a third person administered the injection. Ataxia, lethargy, decreased activity, tremors and unsteady gait were observed in groups receiving atropine alone or in combination with HI-6 dm. Because these CNS effects were only seen in groups receiving ATC, they were considered related to atropine's central anti-muscarinic actions. Emesis was observed in high dose HI-6 dm animals and in low and high combination dose animals. Significant decreases in body weights and/or weight gains accompanied by decreases in food consumption were observed in males receiving atropine alone or in combination with HI-6 dm. Although not statistically significant, decreases in body weights or weight gains accompanied by decreases in food consumption were also seen in females receiving atropine alone and the low dose combination. Decreased food consumption was also observed in high dose HI-6 dm males.

Slight positive chronotropic and dromotropic effects were seen in animals which received atropine with or without HI-6 dm. These ECG changes were associated with atropine's pharmacologic action and were not considered adverse effects. Minimal, but significant increases in serum ALT and/or AST in high dose HI-6 dm animals and high dose combination females, not accompanied by corresponding histopathologic changes, suggests that the high dose of HI-6 dm may be marginally hepatotoxic.

Myotoxicity (subacute inflammation) was observed at the last injection site in all groups including ATC placebo-treated animals (vehicle controls). Increases in serum CK levels were observed in treatment groups receiving HI-6 dm alone or in combination with ATC, but not in vehicle-treated controls or in ATC-treated animals. However, based upon the results of the previously conducted two week co-administration study of HI-6 dm and ATC in rats (UIC/TRL Study No. 132), the myotoxicity observed appears to be mainly resulting from the acidic dosing solutions and not a direct toxic effect of either test article. The addition of HI-6 dm to either the ATC placebo or the ATC dosing formulation notably decreases the pH of the dosing solutions. The observation of myofiber necrosis in one high dose HI-6 dm male and hemorrhage in all high dose HI-6 dm males and in one high dose HI-6 dm female may also be related to the decreased pH seen in dosing solutions of high HI-6 dm alone compared to those also containing ATC (UIC/TRL Study No. 132).

These findings indicate that the anti-cholinergic action of atropine was largely responsible for the majority of the biologic effects (CNS depression with subsequent decreases in body weights and food intake, and ECG changes) observed in combination drug-treated animals. The addition of HI-6 dm to ATC did not appear to increase these anti-cholinergic responses, except in the case of tremors where a slight increased occurrence was seen in dogs receiving the drug combination compared to ATC alone. Emesis, increased myotoxicity (hemorrhage and myofiber necrosis) and marginal hepatotoxicity were observed in high dose HI-6 dm treated animals. Likewise, the addition of ATC to HI-6 did not appear to potentiate these apparent HI-6 dm-induced effects. Therefore, the combination of ATC and HI-6 dm generally did not potentiate the biologic effects seen from each drug given separately, except possibly increased tremors as previously noted.

Study No. 131 (Task Order UIC-8)

This dose range-finding study assessed the toxicity in rats of co-administered HI-6 dichloride monohydrate (HI-6 dm) and atropine citrate (ATC) in the formulation used in the Atropen autoinjector following one week of daily administration by intramuscular injection. The dose levels studied were 0 (ATC placebo), and 150 mg/kg/day of HI-6 dm co-administered with ATC dose levels of 1, 2, 4, 7 or 14 mg base/kg/day. Treatment with the test article dosing solutions produced a painful stimulus as judged by escape behavior and vocalization. The noxious stimuli may possibly account for the observance of rough coat in a few animals in most of the test article-treated groups. Possible decreases in body weight gains were seen in test article-treated males, but not females in an ATC dose-independent manner without accompanying decreases in food consumption. At necropsy, no test article-related gross lesions were observed. One early death (male; HI-6 150 mg/kg/day and ATC 7 mg base/kg/day) occurred on day 4. At necropsy, the animal's airway was obstructed by a large bolus of food, possibly related to atropine's anti-cholinergic effect, i.e. reduction of salivary and esophageal secretions thereby inhibiting the

movement of food through the esophagus. Based upon these findings and after consultation with the Sponsor, an ATC dose level of 14 mg base/kg/day was selected for use in a two week intramuscular toxicity study of co-administered HI-6 dm and atropine citrate in rats.

Study No. 132 (Task Order UIC-8)

This study evaluated the toxicity in rats of co-administered HI-6 dichloride monohydrate (HI-6 dm) and atropine citrate (ATC), the formulation used in the Atropen autoinjector, following two weeks of daily administration by intramuscular injection. The dose levels of HI-6 dm and ATC were 0, 17, 50, or 150 mg/kg/day and 0 or 14 mg base/kg/day, respectively. The control group received the vehicle (ATC placebo) which was the Atropen autoinjector formulation without atropine.

Three deaths in the study (an ATC-treated male, a mid dose combination male and a high dose combination female) were associated with the anti-cholinergic action of atropine producing dysphagia which resulted in asphyxiation due to obstruction of the larynx by boluses of food. Dysphagia was also observed in other animals receiving atropine with or without HI-6 dm, but not in any animal receiving HI-6 alone or in control animals. The administration of the dosing solutions including the ATC placebo produced a painful stimulus as judged by escape behavior and vocalization, resulting in stress-induced poor grooming and slight leukocytosis in all drug-treated and control groups. A significant decrease in body weight gains accompanied by a decrease in food consumption was observed once in high dose combination males, but did not result in statistically significant decreases in body weights or total weight gains, and was not observed in females. Hunched posture was also observed in one high dose combination male.

Myotoxicity (subacute inflammation, myofiber degeneration and hemorrhage) was observed at the last injection site in ATC placebo-treated animals (vehicle controls) and in high dose combination animals to a similar extent. These lesions were considered to be related to the large dosing volume being repeatedly administered and/or the physical properties of the dosing solutions (pH or tonicity). Mild thrombocytosis was seen in all groups including vehicle-treated control animals, and was apparently secondary to the myotoxicity (hemorrhage) induced by the intramuscular injections.

Minimal, but significant increases in serum ALT, AST and/or LDH in mid and high dose combination females, not accompanied by corresponding histopathologic changes, suggests that joint test article treatment may be marginally hepatotoxic. The increased AST and LDH levels may also be related to the observed myotoxicity. It is not known why these changes were not apparent in HI-6 dm alone-treated animals, as previously seen (UIC/TRL Study No. 072). Additionally, increased serum total bile acids were seen in ATC-treated animals, either alone or in combination with HI-6 dm (mid dose combination animals and high dose combination males but not females), but not in low dose combination animals or any group treated with HI-6 dm alone. These apparent changes in hepatobiliary function may be related to atropine's anti-spasmodic action on the smooth muscle of the bile ducts. Because toxicity was observed in mid and high dose combination groups, the drug combination no observed effect level (NOEL) in the present study was determined to be 17 mg/kg/day HI-6 dm/14 mg base/kg/day ATC.

Study No. 219 (Task Order UIC-9)

The purpose of this study was to determine specific target organ toxicity, dose-reponse relationships, and a no observed adverse effect level of WR238605 succinate in Beagle dogs following one year of daily oral administration. WR238605 succinate is being developed as an antimalarial agent. Dose levels studied were 0, 0.1, 1.0 and 4.0 mg base/kg/day. The primary toxicities of WR238605 succinate following one year of oral administration were to the lungs and red blood cells. No mortalities occurred in the study. Body weight gains were decreased in the males in a dose-dependent fashion, but were unaffected in females. Clinical signs were primarily seen in the mid and high dose groups and included diarrhea, emesis and blue tongue. Increased respiratory rate was observed in one high dose male. Methemoglobinemia was produced throughout the study in the mid and high dose groups. Chronic, low level intravascular hemolysis occurred in the mid and high dose groups, as evidenced by the presence of tissue pigmentation changes in Kupffer cells, renal cortex epithelium and in macrophages in spleen, gall bladder, tonsil and lymph nodes (mesenteric, mandibular, bronchial and mediastinal). Furthermore, increased reticuloctye counts, Heinz bodies and serum haptoglobin levels were seen. Thrombocytopenia was seen in the high dose group in week 4, but resolved thereafter. Pulmonary lesions were observed in all animals in the mid and high dose groups, and consisted of foamy macrophage accumulation and chronic interstitial inflammation. Bone marrow hyperplasia occurred in the mid and high dose groups. Lung, liver and splenic weights were increased at the high dose level. Although subtle ECG changes were seen and appear to be treatment-related, they may not represent significant toxicologic effects. A no-effect level was considered to be at or near the low dose of 0.1 mg base/kg/day.

Study No. 153 (Task Order UIC-10)

This dose range-finding study evaluated the developmental toxicity of WR238605 Succinate in time-mated CD® female rats. Doses were 0, 1, 2.5, 6, 15 and 35 mg base/kg/day administered by gavage during gestation days (GD) 6 - 15 (GD0 = day of vaginal plug).

At the 35 mg base/kg/day dose, body weights were significantly decreased starting on GD9. This was reflected as a significant decrease in total weight gain by GD20. A marginal biological decrease in body weights was also observed at the 15 mg base/kg/day dose. Food consumption was significantly decreased at the intervals GD6-10, 10-15 and 15-20 at the 35 mg base/kg/day dose, while at the 15 mg base/kg/day dose, significant decreases were only observed during the second half of dosing i.e. GD10 through 15. No mortality was observed at any group, however rough coat was seen in two animals in the high and in two animals in the midhigh doses. Accordingly, the 15 mg base/kg/day dose was considered at or near the maternal low observable adverse effect level (LOAEL).

In fetuses, decreases in body weights were observed in the 15 and 35 mg base/kg/day doses. These decreases were statistically significant in females, while males showed statistical significance only in the high dose. With the exception of one low dose fetus demonstrating spurious anomalies, no significant change in the incidence of external anomalies was observed in any dose group when compared to the control. Normal variations (hematomas and petechial hemorrhages) were observed in all the dose groups without any dose-response relationship. The

numbers of early and late resorptions in various groups were not representative of any test article-related effect. The 15 mg base/kg/day was considered a marginal developmentally toxic dose. Accordingly, the following doses are suggested for the definitive developmental toxicity (segment II) study in rats: 0, 3, 10 and 30 mg base/kg/day.

Study No. 154 (Task Order UIC-10)

The objective of this study was to evaluate the potential developmental toxicity of WR238605 Succinate in time-mated CD® female rats. WR238605 Succinate is an 8-aminoquinoline derivative which has demonstrated antimalarial potential in preclinical studies. Doses used in this study were 0, 3, 10, and 30 mg base/kg/day and were based on a dose range-finding study (UIC/TRL Study No. 153) in which maternal and fetal toxicity were seen at 35 and to a lesser extent at 15 mg base/kg/day. The drug or vehicle was administered by gavage to 25 rats/group during gestation days (GD) 6 - 15 (GD0 = day of vaginal plug). In addition, a positive control group of 25 animals was administered retinol palmitate, 1000 mg/kg/day, on GD9 and 10 by gavage. At dosing initiation (GD6), the animals were 69 - 71 days old and weighed 219 - 267 g.

Maternal toxic manifestations were observed in the high dose and to a lesser extent in the mid dose and positive control groups . A significant decrease in body weight from GD8 resulting in a reduction in total weight gain was associated with a significant decrease in food consumption in high dose females (i.e., 30 mg base/kg/day). Mid dose females (i.e., 10 mg base/kg/day) showed significant decreases in body weight and food consumption only during the period of dosing (i.e., GD6 - 15). Other toxic manifestations were limited to rough coat in two animals at the high dose and enlarged spleen in 16 high dose and 1 mid dose animals. Toxicity was not observed in the low dose level. The positive control group showed a significant decrease in body weight and total weight gain from GD10 - GD20, in addition to a decrease in food consumption during the dosing period (i.e., GD6 - GD15).

The doses of WR238605 succinate tested in this study did not result in any developmental toxicity. The 3 mg base/kg/day dose was considered the maternal no observable effect level (NOEL) while 30 mg base/kg/day, the highest dose tested, was considered the NOEL for fetal toxicity in rats. Neither maternal nor fetal effects were seen in the vehicle animals. Significant developmental toxic manifestations were, however, seen in the positive control group. These manifestations included significant decreases in the number of viable fetuses, fetal body weights and maternal uterine weights. In addition, significant increases in % postimplantation loss, and increased incidences in fetal skeletal and visceral malformations, primarily related to structures of the head, vertebral column and ribs, were noted in retinol palmitate-treated animals.

Study No. 155 (Task Order UIC-10)

This dose range-finding study evaluated the developmental toxicity of WR238605 succinate in time-mated New Zealand White (Pasteurella Free) female rabbits. Doses were 0, 2, 4, 8, 16 and 32 mg base/kg/day administered by gavage during gestation days (GD) 6 - 18 (GD0 = day of observed mating). The doses were based on a preliminary dose range-finding test of WR238605 succinate in non-pregnant rabbits and a dose range-finding developmental toxicity study in rats.

Maternal toxic manifestations were only obvious at the high dose (i.e., 32 mg base/kg/day). Two animals aborted after the end of the dosing period. In addition, significant decreases in food consumption from GD12 and in weight gain from GD16 were present. The incidence of postimplantion loss was significantly increased due to a biological decrease in the number of viable fetuses and an increase in early resorptions. The 16 mg base/kg/day dose level was considered the maternal NOEL. Fetal toxicity was also obvious at the high dose as a statistically significant decrease in body weights. Reductions in fetal body weights may have also occurred to a limit extent at 8 and 16 mg base/kg/day. The 8 mg base/kg/day dose was considered the NOAEL dose for fetal toxicity.

The results obtained in the current study and in a previous dose range-finding of WR238605 succinate in rats (UIC/TRL No. 153) demonstrated similar sensitivity of rabbits and rats to the test article and suggested direct developmental toxicity. Accordingly, the following doses were chosen for the definitive developmental (Segment II) toxicity study of WR238605 succinate in rabbits; 0, 2, 7 and 25 mg base/kg/day.

Study No. 156 (Task Order UIC-10)

This study evaluated the embryo/fetal toxicity and the teratogenic potential of WR238605 Succinate in time-mated New Zealand White (Pasteurella Free) female rabbits. WR238605 Succinate is an 8-aminoquinoline derivative which has demonstrated antimalarial potential in preclinical studies. Doses were based on the results of a previously conducted dose range-finding study (UIC/TRL Study No.155) where maternal toxicity (abortion, significant reductions in body weight and food consumption, and a significant increase in postimplantation loss due to a biological decrease in the number of viable fetuses and an increase in early resorptions) was evident at 32 mg base/kg/day and fetotoxicity (a significant decrease in body weight) was noted at the 32 mg base/kg/day dose level and to a lesser extent at the 16 and 8 mg base/kg/day dose levels.

In the present study, doses of 0, 2, 7, or 25 mg base/kg/day were administered by gavage to 20 rabbits/group during gestation days (GD) 6 - 18 (GD0 = day of observed mating). In addition, a positive control group of 20 rabbits was administered retinol palmitate, 300 mg/kg/day, on GD9 and 10 by gavage. Due to an apparent dosage formulation analysis problem, the high dose was retested at the Sponsor's request. Doses of 0 or 25 mg base/kg/day were administered by gavage to 20 rabbits/group during GD6 - 18. Due to mortality and reductions in food consumption, the dose was lowered to 16 mg base/kg/day on GD15 - 16 (the range of days reflects study stagger-start over two days). Animals in both aspects of the study were 6 - 6½ months old at the initiation of dosing (GD6). The weight range at the initiation of dosing (GD6) was 2.62 - 3.75 kg in the initial study and 2.96 - 4.33 kg in the retest of the high dose. Terminal necropsies were conducted on GD29.

Maternal toxicity was observed in the initial 25 mg base/kg/day group, and included abortion (2/20) and premature delivery (1/20). A significant loss in body weight gain on GD9 contributed to a slight, but insignificant reduction in total body weight gain. Statistically significant decreases in food consumption were due to effects in these few animals. Maternal toxicity was also

observed in the retest 25/16 mg base/kg/day group as exemplified by mortality (2/20), moribund sacrifice (2/20), and abortion (3/20). Statistically significant decreases in body weight and food consumption were attributed to reductions noted in the majority of animals in this retest group. There were no alterations in maternal reproductive indices or fetal parameters in any of the initial or retest WR238605 Succinate-treated groups.

One rabbit at 7 mg base/kg/day aborted but showed no other evidence of maternal toxicity. Based on the incidence of abortion in the 25 and 25/16 mg base/kg/day groups, this event may be related to administration of WR238605 Succinate. No evidence of maternal toxicity was seen in the 2 mg base/kg/day group. At the request of the Sponsor, skeletal evaluations were not conducted in fetuses in the initial 25 mg base/kg/day group. There was no embryotoxicity, fetotoxicity, or teratogenicity at any dose level. Based on the results of this study, the no-effect level (NOEL) for maternal toxicity of WR238605 Succinate in pregnant rabbits was at or near 7 mg base/kg/day, and the fetal NOEL was 25 mg base/kg/day.

Neither maternal nor fetal affects were seen in the vehicle control animals. Fetotoxicity and teratogenicity were noted in the positive control group. Slight, nonstatistically significant reductions were noted in uterine weight and fetal viability. A statistically significant increase in the number of early resorptions was accompanied by a nonsignificant reduction in the number of viable fetuses as well as nonsignificant increases in the percent postimplantation loss and total loss/litter. Statistically significant increases in the incidence of external, skeletal, and visceral fetal malformations were considered typical of retinol palmitate (e.g., cleft palate, micrognathia, microstomia). In addition, a statistically significant increase was noted in major blood vessel variations and the incidence of distended ureter.

Task Order UIC-10

WR238605 Succinate was tested for point mutations and chromosomal aberrations in three *in vitro* mutagenicity tests conducted by Microbiological Associates, Inc. via a subcontract mechanism. WR238605 Succinate was shown to be negative in the chromosomal aberrations test but was equivocal in the mouse lymphoma assay, which tested for point mutations. Accordingly, a second *in vitro* test for point mutations, the CHO/HGPRT assay, was performed. WR238605 Succinate was negative in this follow-up point mutation assay, and a subsequent confirmatory *in vivo* mutagenicity test is, therefore, not warranted. Accordingly, the test article does not appear to represent a genotoxic hazard.

Study No. 166 (Task Order UIC-11)

This study evaluated the toxicity of halofantrine hydrochloride in B6C3F1 mice following thirteen weeks of daily oral (gavage) administration. Halofantrine HCl is being developed by WRAIR as an oral antimalarial treatment. Dose levels studied were 0 (vehicle control), 1, 5 and 25 mg/kg/day, and were based on a four week dose range-finding test in which mortality occurred at 100 mg/kg/day with anemia present at 20 mg/kg/day and to a minimal extent at 4 mg/kg/day. The drug or vehicle was administered to 10 mice/sex/group. The animals were ≈ 8 weeks old and weighed 23.0 - 25.8 g (males) and 17.7 - 21.7 g (females) at treatment initiation.

No clinical signs of toxicity were observed and body weight gains and food consumption were not affected by test article treatment. Treatment-related ophthalmic changes were also not observed. The primary treatment-related effects of halofantrine were microcytic anemia and marginal changes in the liver. An increased severity and/or incidence of hepatic glycogen depletion was observed in high dose animals compared to control mice. In addition, individual cell necrosis was seen in two high dose females but not males. These marginal liver changes were accompanied by statistically insignificant increased serum ALT levels and decreased total protein and albumin levels in high dose males. Marginal treatment-related anemia, including decreases in hemoglobin, hematocrit, mean corpuscular volume (MCV) and/or mean corpuscular hemoglobin (MCH), in the absence of a reduction in RBC count, was observed in high dose animals. These microcytic anemic changes without corresponding compensatory responses or histopathologic changes in bone marrow are suggestive of an iron-deficiency anemia. Very slight but statistically significant microcytosis was also seen in mid dose males, but was not considered biologically significant.

The no-observed effect level (NOEL) in the present study was considered to be at or near 5 mg/kg/day. This study was conducted to select dose levels for a subsequent two year carcinogenicity study in mice. Because marginal halofantrine-induced toxicity including anemia and mild liver toxicity was seen in high dose animals (25 mg/kg/day), the following dose level ranges are suggested: 2 - 4 , 7 - 12 and 25 - 35 mg/kg/day. Accordingly, dose levels of 3, 10 and 30 mg/kg/day are recommended.

Study No. 168 (Task Order UIC-11)

This dose range-finding study evaluated the toxicity of halofantrine hydrochloride in B6C3F1 mice following four weeks of daily oral (gavage) administration. Dose levels studied were 0 (vehicle control), 4, 20 and 100 mg/kg/day. Clinical signs of toxicity (rough coat, hunched posture, decreased activity and lethargy) and decreased body weight gains were limited to high dose animals. During week 3, one high dose male was found dead and the other four high dose animals were sacrificed moribund. Splenic lymphocytic necrosis, observed in all high dose males and three of five high dose females, and moderate splenic lymphocytic depletion, were considered possible contributing factors to their deaths. Splenic granulopoiesis secondary to the splenic lymphocytic necrosis, supported by neutrophilia and splenomegaly, was observed in high dose females. Marginal leukopenia, consisting of decreased numbers of mature neutrophils and lymphocytes, was seen in mid dose males but not females and may be indicative of the initial insult producing splenic lymphocytic depletion in the high dose animals. Dose-related, mild, microcytic, apparent iron-deficiency anemia was seen in high dose females and to a lesser extent in mid dose animals and low dose females. Thrombocytosis in high dose females may have been secondary to the anemia. Increased serum ALT and cholesterol levels in high dose females and increased serum ALT in mid dose males, not accompanied by corresponding histologic changes, suggests that halofantrine may be marginally hepatotoxic. Decreases in serum alkaline phosphatase levels were also observed in high dose females, and may have been related to reductions in food intake. The purpose of the study was to select dose levels for a three month toxicity study in mice. Because marginal halofantrine-induced toxicity was seen in low dose females, the following dose level ranges are suggested: 1 - 2, 4 - 8 and 15 - 30 mg/kg/day.

Study No. 176 (Task Order UIC-12)

This study evaluated the local and systemic (organ) toxicity of WR279396 (Iowa Formulation 232 containing 15% paromomycin sulfate and 0.5% gentamicin sulfate) in CD® rats following four weeks of daily dermal application. Three groups, each composed of 10 male and 10 female rats, were initially given the test article twice daily by dermal application for the first five days. The volume of WR279396 administered per application (2 applications/day) were 0.07, 0.33, and 1.67 ml/kg, respectively, in low, mid and high dose animals. This corresponds to doses of 20, 100 and 500 mg/kg/day of paromomycin + 0.7, 3.3, 16.7 mg/kg/day of gentamicin, respectively. A control group of 10 male and 10 female rats received the test article vehicle (WR279396-Placebo, Iowa Formulation 232) by dermal application at a dosing volume of 1.67 ml/kg per application. Due to the appearance of moderate to severe erythema in mid and high dose animals on days 4 and 5, the volume (amount) of test article or vehicle control article administered was reduced to one-half the initial dose levels beginning on day 6 and for the remainder of the study. On day 6 and thereafter, test article volume (amount) was administered once daily in the morning instead of as a split dose twice daily.

Following reduction in dosing frequency, very slight erythema (draize score = 1, barely perceptible), not accompanied by edema formation, was seen in most high dose animals. Acanthosis, thickening of the stratum spinosum layer of the epidermis, was also observed in several high dose animals and was generally of minimal severity. Except for one mid dose male, these dermal histologic changes were not seen at lower dosing volumes of WR279396, *i.e.* 0.07 or 0.33 ml/kg/day (dose of 20 and 100 mg/kg/day of paromomycin + 0.7 and 3.3 mg/kg/day of gentamicin, respectively). Clinical signs, body weights, food intake, clinical chemistry and hematology parameters, ophthalmology evaluations and organ weights were not affected by test article treatment in any of the dose levels tested. A no-observed effect level (NOEL) was considered to be at or near 0.33 ml/kg/day of WR279396 administered once daily, corresponding to 50 mg/kg/day paromomycin + 3.3 mg/kg/day gentamicin in Iowa Formulation 232. However, more frequent application of test article per day results in dermal irritation and potentially histologic changes in the skin at the exposure site. Following six days of treatment, the twice daily application of the volume of test article produced well-defined erythema in low dose animals and moderate to severe erythema in mid and high dose animals.

Study No. 182 (Task Order UIC-12)

This dose range-finding test to select doses for a 4 week dermal toxicity study of WR279396 in rats was cancelled by the Sponsor.

Study No. 170 (Task Order UIC-13)

This dose range-finding study evaluated the developmental toxicity of WR6026 Dihydrochloride in time-mated CD® female rats. Doses were 0, 1.5, 3, 6, 12 and 24 mg base/kg/day administered by gavage during gestation days (GD) 6 - 15 (GD0 = day of vaginal plug). Maternal toxicity was mainly observed at 24 mg base/kg/day and consisted of rough coat on sporadic occasions in three animals, significant decreases in food consumption (GD10 through 15), and sporadic decreases

in body weight during the dosing period. At 12 mg base/kg/day, rough coat was observed in 3 animals around GD8 - 13. The 12 mg base/kg/day dose was considered at or near the maternal no observable effect level (NOEL). Fetal toxicity was seen as a significant decrease in fetal body weights at 24 mg base/kg/day. No other developmental or maternal toxicity parameters were affected at any dose level. Accordingly, 2, 8 and 30 mg base/kg/day are suggested for the main developmental toxicity (Segment II) study in rats to elicit maternal toxicity at the high dose and to avoid developmental toxicity at the low dose.

Study No. 171 (Task Order UIC-13)

This study evaluated the embryo/fetal toxicity and the teratogenic potential of WR6026 Dihydrochloride in CD® female rats. WR6026 Dihydrochloride is being developed for oral treatment of visceral leishmaniasis. Doses were 0, 2, 8, and 30 mg base/kg/day and were based on a dose range-finding study (UIC/TRL Study No. 170) in which maternal and fetal toxicity were seen at 24 mg base/kg/day with minimal maternal effects observed at 12 mg base/kg/day. The drug was administered daily by gavage during gestation days GD6 - 15 (GD0 = day of vaginal plug). In addition, a positive control group was administered retinol palmitate, 1000 mg/kg/day, on GD9 and 10 by gavage. Maternal toxic manifestations were observed mainly in the high dose group and to a lesser extent in the mid dose. In the high dose group, 4 females were found dead between GD14 and 18 and one was sacrificed moribund on GD14. Almost all females in this dose showed rough coat and pale appearance that lasted from 2 - 10 days. Some animals also showed hunched posture and dark material around the nose. Significant decreases in weight gain and food consumption were also apparent from GD6 through GD20. At necropsy, splenic enlargement in 13 animals was observed. Mid dose females showed a significant decrease in food consumption on GD6 - GD15 associated with a significant decrease in total weight gain. The only maternally significant toxic manifestation in the low dose was a transient decrease in food consumption during GD6 - 10. Accordingly, 2 mg base/kg/day was considered at or near the maternal no observable effect level (NOEL).

Fetal toxicity was observed as significant decreases in fetal body weight in the mid and high dose levels. A significant increase in the number of litters with reduced ossification of the cervical arches was also seen in the high dose. This developmental variation was probably secondary to retarded development (observed as reduced fetal body weights). A marginal, statistically insignificant decrease in fetal body weight was also observed in the low dose. No other fetal toxic manifestations were observed. Accordingly, 2 mg base/kg/day was considered at or near the fetal NOEL.

In the positive control group, maternal and fetal toxicity were observed. Maternal toxicity manifestations included rough coat in two females; significant reductions in weight gain, uterine weight and food consumption during GD10 - 15; and increases in early resorptions, % post implantation loss and % total implantation loss. Fetal toxicity was observed as significant decreases in fetal body weight, decreases in the number of viable fetuses, and increases in the incidences of external and skeletal malformations, primarily related to structures of the head, vertebral column and ribs.

Study No. 172 (Task Order UIC-13)

This dose range-finding study evaluated the developmental toxicity of WR6026 dihydrochloride in time-mated New Zealand White (Pasteurella Free) female rabbits. Doses were 0, 1, 2, 4, 10 and 20 mg base/kg/day administered by gavage during gestation days (GD) 6 - 18 (GD0 = day of observed mating). The doses were based on a preliminary dose range-finding test of WR6026 dihydrochloride in non-pregnant rabbits.

Maternal toxicity was mainly observed in 2 animals in the high dose group. These females showed rapid respiration, blue sclera and pallor of the lips and ears followed by mortality in one animal on GD11 and a decrease of ≈ 15% in body weight in the other animal, which was subsequently sacrificed moribund on GD15. In addition, marginal decreases in food consumption (GD8 through 15) and in total weight gain were observed in the same dose. No other drug-related effects were observed in any dosed group or on any maternal reproductive indices in any dose. Accordingly, the 10 mg base/kg/day dose was considered the reproductive no observable effect level (NOEL). No significant manifestations of fetal toxicity were observed, however marginal, biological reductions in female fetal body weights were apparent in the high dose. The 10 mg base/kg/day dose level was therefore considered the no observable effect level for fetal toxicity. Accordingly, the following doses were selected for the developmental (Segment II) definitive toxicity study of WR6026 dihydrochloride in rabbits; 0, 3, 7 and 15 mg base/kg/day.

Study No. 173 (Task Order UIC-13)

This study evaluated the developmental toxicity of WR6026 Dihydrochloride in time-mated New Zealand White (Pasteurella Free) female rabbits. WR6026 Dihydrochloride is being developed as an oral teatment for visceral leishmaniasis. Doses were 0, 3, 7, and 15/10 mg base/kg/day and were based on a dose range-finding study (UIC/TRL Study No. 172) in which maternal toxic effects including lethality and statistically insignificant decreases in fetal body weights were seen at 20 mg base/kg/day, but no changes were noted at 10 mg base/kg/day. The drug was administered by gavage during gestation days (GD) 6 - 18 (GD0 = day of observed mating). The high dose was reduced from 15 mg base/kg/day to 10 mg base/kg/day on gestation days 12 - 15 due to maternal lethality (the range of days reflects study stagger-start over 4 days). A positive control group was administered retinol palmitate, 300 mg/kg/day, by gavage on GD9 and GD10 or GD9 and GD12 (in 5 females). Maternal toxic manifestations were only observed in high dose animals. After four of 20 females in the high dose group died during the first several days of treatment, the high dose was reduced to 10 mg base/kg/day. Increases in respiratory rate associated with bluish discoloration of the sclera and ears in some animals were seen for intervals of 1 - 11 days during the dosing period (i.e., GD6 - 18). Statistically significant sporadic reductions in body weight/weight gain and food consumption were observed prior to high dose reduction. No drug-related changes in any maternal reproductive indices were observed. In the previously-conducted dose range-finding study in rabbits (UIC/TRL Study No. 172), no maternal toxicity was observed at 10 mg base/kg/day although mortality did occur at the 20 mg base/kg/day dose level.

No evidence of developmental toxicity was observed at the 3 and 7 mg base/kg/day levels.

Teratologic results at the maternally lethal dose of 15/10 mg base/kg/day were equivocal as vertebral malformations were observed in two litters, the incidence of which was not statistically significant. The no-effect level for developmental toxicity of WR6026 Dihydrochloride in rabbits was at least 7 mg base/kg/day and possibly 15/10 mg base/kg/day.

Females in the positive control group did not show any toxic manifestations, however, biologically marginal decreases in the number of viable fetuses resulted from statistically significant increases in the number of early resorptions, % post implantation loss and % total loss/litter. Significant increases in the incidence of external, visceral and skeletal malformations were also noted in these litters.

Task Order UIC-14

WR6026 Hydrochloride was tested for point mutations and chromosomal aberrations in three *in vitro* mutagenicity tests conducted by Microbiological Associates, Inc. via a subcontract mechanism. WR6026 Hydrochloride was negative in the Ames Test, the Mouse Lymphoma Assay, and the Chromosome Aberration Test using Chinese Hamster Ovary cells.

Study No. 152 (Task Order UIC-15)

This study evaluated the toxicity of WR238605 Succinate in rats following six months of daily oral (gavage) administration. WR238605 Succinate is an 8-aminoquinoline derivative which has demonstrated antimalarial potential in preclinical studies. Dose levels studied were 0 (vehicle control), 0.5, 2.0 and 9.0 mg base/kg/day, and were based on a three month toxicity study with a three month recovery period in rats (UIC/TRL Study No. 098) in which anemia and lung lesions were seen at 6 and 18 mg base/kg/day whereas 0.5 mg base/kg/day was the no-observed effect level.

The primary toxicities of WR238605 Succinate were to RBCs, the lungs and the liver. Mortality occurred in one high dose male rat. Treatment-related clinical signs in high dose animals included rough coat, hunched posture, labored breathing (males), and piloerection (females). Body weight gains were significantly reduced in high dose animals and mid dose males. Also, food consumption was decreased in high dose animals. High dose males, and mid and high dose females had decreased RBC counts, HCT and HGB concentration, suggestive of mild anemia. The anemia may have been hemolytic in origin due to the presence of Heinz bodies and increased methemoglobin levels. Microscopic lesions observed in the spleen, bone marrow, kidneys and adrenal glands may have been secondary to anemia and/or hemolysis. High dose animals had elevations in mature neutrophil and lymphocyte numbers. Mild thrombocytopenia was seen in mid and high dose males. Pulmonary lesions in male and female rats in the mid and high dose groups consisted of foamy macrophage accumulation, chronic interstitial inflammation, and hemorrhage (high dose groups only). Apoptosis, pigmentation and fatty change in the centrilobular region of the liver were seen in high dose males, but not in females, and were accompanied by decreased serum globulin, and BUN, and increased total bile acids. Similar clinical chemistry changes were seen in high dose females. The no-effect level (NOEL) for WR238605 Succinate is considered to be at or near the low dose of 0.5 mg base/kg/day.

Study No. 185 (Task Order UIC-15)

This study to determine the pharmacokinetics of WR238605 in rats was cancelled by the Sponsor.

Study No. 197 (Task Order UIC-16)

This study evaluated the toxic potential of WR242511 Tartrate on reproductive capability in CD® male and female rats. WR242511 Tartrate is being developed as an anticyanide agent. Doses were 0, 0.3, 1.0, and 3.0 mg base/kg/day and were based on a three month toxicity study in male and female rats (UIC/TRL Study No. 107) in which mortality and toxicity was seen in males at doses of 4.5 mg base/kg/day and on a developmental toxicity study in female rats (UIC/TRL Study No. 144) in which maternal toxicity was observed at 8 mg base/kg/day.

In the present study, doses of 0, 0.3, 1.0, and 3.0 mg base/kg/day were administered by daily gavage to male CD® rats for at least 60 days and to pregnant female CD® rats for 23 - 27 days in sperm-positive animals and for 48 days in sperm-negative females. This included 29 days of dosing prior to cohabitation in males and 15 days of dosing prior to cohabitation in females. Lethality/toxicity was seen primarily in the high dose males, while only toxicity occurred in high dose females. Six of 25 high dose males were sacrificed moribund on day 15 or 21. Clinical signs in these and/or the remaining high dose animals included dark material around the nose, piloerection, hunched posture, and rough coat. Significant reductions in body weights, body weight gains, and food consumption occurred in males primarily at 3 mg base/kg/day. In males at 1 mg base/kg/day, sporadic, nonsignificant reductions in body weight gains (in the absence of body weight loss) contributed to a significant reduction in total body weight gains. Decreased organ weight to brain weight ratios were noted in high dose males for the epididymis, seminal vesicles, and prostate. However, there were no effects on sperm motility, count, or morphology in any group, and no apparent effects on the males' ability to impregnate the females.

Females in the 3 mg base/kg/day group exhibited significantly reduced body weights and food consumption during the precohabitation and gestation periods. There were no effects on Cesarean section or estrous cycle parameters in any group. Both the mating and fertility indices were 100% in all WR242511 Tartrate groups. The no-observable-effect level (NOEL) for reproductive capability of males and females was 3 mg base/kg/day in spite of lethality and/or toxicity noted in both sexes at this dose level.

Study No. 193 (Task Order UIC-18)

This study evaluated the toxicity of WR242511 tartrate in male and female beagle dogs following thirteen weeks of daily oral administration by gelatin capsule. A thirteen week recovery period was included for all groups. WR242511 tartrate is being developed as an anticyanide agent. Dose levels studied were 0, 0.1, 0.3 and 1.0 mg base/kg/day and were based on a one month toxicity study in beagle dogs in which 1.0 mg base/kg/day resulted in toxicity to RBCs, lungs and platelets and a no-observed effect level (NOEL) of 0.1 mg base/kg/day was seen (UIC/TRL Study No. 134). The dogs were ≈ 7 - 8 months old and weighed 10.3 - 13.6 kg (males) and 7.4 - 11.3 kg (females) at dosing initiation.

In the present investigation, the primary toxic effects of WR242511 tartrate were seen in the lungs, RBCs and platelets at the 0.3 and 1.0 mg base/kg/day dose levels. Mild reductions in body weight gain were seen in mid and high dose animals. Methemoglobin, the desired pharmacologic effect, was produced in a dose-dependent fashion and was accompanied by clinical signs of cyanosis (blue gums, tongue and sclera). Mild anemia accompanied by reticulocytosis, secondary splenic hematopoiesis and bone marrow hyperplasia occurred primarily in high dose animals. Significant thrombocytopenia was seen during the treatment period in the mid and high dose animals. Administration of WR242511 resulted in pulmonary lesions in mid and high dose animals consisting of alveolar macrophage accumulation, chronic perivascular inflammation, chronic interstitial inflammation, and basophilic granular material in the alveoli. Minimal, but statistically significant changes in clinical chemistry parameters suggestive of liver injury were seen. However, histopathologic evidence of liver injury was not observed, suggesting that WR242511 is marginally hepatotoxic. By the end of the 13 week recovery period, treatment-related effects had resolved except for treatment-related pulmonary lesions that were of such minimal severity as to be considered biologically insignificant. Because the aforementioned toxic responses were limited to the mid and high dose levels, the no-observed effect level (NOEL) of WR242511 tartrate was 0.1 mg base/kg/day.

Study No. 199 (Task Order UIC-20)

This study evaluated the toxic potential of WR238605 Succinate on reproductive capability in CD® male and female rats. WR238605 Succinate is being developed as an antimalarial agent. Doses were 0, 1.5, 5.0, and 15 mg base/kg/day and were based on a three month toxicity study with a three month recovery period in male and female rats (UIC/TRL Study No. 098) in which decreased body weight gains and food consumption as well as enlarged spleens, alveolar proteinosis, methemoglobin production, and mild anemia were observed in both sexes at 6 and 18 mg base/kg/day; and on a developmental toxicity study in female rats (UIC/TRL Study No. 154) in which maternal toxicity was observed at 10 and 30 mg/kg/day.

In the present study, doses of 0, 1.5, 5.0, and 15 mg base/kg/day were administered by daily gavage to male CD® rats for at least 67 days and to pregnant female CD® rats for 23 - 28 days in sperm-positive animals and for 47 days in sperm-negative females. This included 29 days of dosing prior to cohabitation in males and 15 days of dosing prior to cohabitation in females.

Lethality/toxicity was seen in the high and mid dose males, while toxicity occurred primarily in high dose females. One of 25 high dose males, found dead on day 28, had an enlarged spleen. Clinical signs in this and/or the remaining males at 15 mg base/kg/day included piloerection, rough coat; and/or audible breathing. Piloerection and rough coat were also observed in males at 5 mg base/kg/day. Significant reductions in body weights and food consumption occurred in males at 5 and 15 mg base/kg/day. Relative weights for the seminal vesicles and prostate were reduced in the high and mid dose males. There were no effects on sperm motility, count, or morphology in any WR238605 Succinate-treated group, and no apparent effects on the males' ability to impregnate the females. Females in the 15 mg base/kg/day group exhibited piloerection, rough coat, and/or increased respiratory rate during the precohabitation/cohabitation and/or gestation phases; enlarged spleens were noted at necropsy. Significantly reduced body weights

and food consumption occurred during the precohabitation and gestation phases. The numbers of *corpora lutea* and, consequently, the number of implantations, and viable fetuses were significantly reduced at 15 mg base/kg/day. There were no effects on estrous cycle parameters in any group; mating and fertility indices were also unaffected by treatment with WR238605 Succinate. This suggests that administration of 15 mg base/kg WR238605 Succinate for 15 days prior to mating affected oocyte maturation but not ovulation, mating behavior, implantation or embryonic development. The no-observable-effect-level (NOEL) for reproductive capability of males was 15 mg base/kg/day in spite of lethality and/or toxicity observed at this dose level. Based on the finding of altered maturation of oocytes at the high dose, the NOEL for females was 5 mg base/kg/day even though mating and fertility indices were unaffected at any dose level.

Study No. 200 (Task Order UIC-21)

This study evaluated the toxic potential of WR238605 Succinate on the pregnant/lactating female CD® rat (F_0 generation) and the survival and development of their offspring (F_1 generation) consequent to exposure from implantation through weaning. Doses were 2, 6, and 18 mg base/kg/day based on a developmental toxicity study in female CD® rats (UIC/TRL Study No. 154) in which decreased body weight gains and food consumption occurred throughout the study at 30 mg base/kg/day while marginal reductions in body weight and food consumption were noted during the dosing period at 10 mg base/kg/day.

In the present study, doses of 2, 6, and 18 mg base/kg/day were administered by daily gavage to female CD® rats (the F_0 generation) for at least 36 days: from gestation day (GD) 0 through postnatal day (PND) 20. The results are summarized in Table 1. There were no mortalities or treatment-related clinical signs or necropsy observations noted in the F_0 generation maternal animals at any dose level. At 18 mg base/kg/day, maternal toxicity was observed as significantly reduced body weights, noted essentially throughout the dosing period (i.e., GD9 - PND21), and significantly reduced food consumption, noted over the gestation period (i.e., GD6 - 20). Significantly reduced food consumption occurred at 6 mg base/kg/day following the initiation of dosing over GD6 - 9; however, body weights were unaffected. Administration of WR238605 Succinate did not affect food consumption or body weights at the low dose. Gestation duration, parturition, and litter size were unaffected by treatment at any dose level. Thus, administration of WR238605 Succinate did not adversely affect the dams' ability to deliver and rear her offspring.

Offspring at 18 mg base/kg/day had evidence of growth retardation and slight developmental and functional delays. Adverse findings included significantly reduced body weights in both sexes throughout the pre- and postweaning periods; slight, significantly delayed attainment of eye opening in both sexes; and slight, but significantly decreased rearing activity in females. All other developmental parameters and neuromotor assessments, survival, and attainment of sexual maturity were unaffected at the high dose. No treatment-related effects occurred in any parameter in the F_1 generation at 2 or 6 mg base/kg/day. In conclusion, the no-observable-effect level (NOEL) of WR238605 Succinate on pregnancy, parturition, and lactation in the F_0 generation dams was 18 mg base/kg/day in spite of toxicity observed at this dose level. Based on alterations in body

weights, and slight developmental and functional delays at the high dose, the NOEL for the development of the F_1 generation was 6 mg base/kg/day.

Task Order UIC-23

Pyridostigmine Bromide was tested for point mutations and chromosomal aberrations in one *in vivo* and four *in vitro* mutagenicity tests. Pyridostigmine bromide was negative in the Bacterial Reverse Mutation Assay, the Chromosome Aberrations Test using Chinese Hamster Ovary cells, and the Micronucleus Cytogenetic Assay in mice. In the Mouse Lymphoma Assay, the test was negative in the absence of hepatic microsomal enzymes, but was positive when these drug metabolizing enzymes were included. A CHO/HGPRT assay, subsequently performed to investigate further the potential to induce gene mutations, was negative. Thus, pyridostigmine bromide does not appear to represent a genotoxic hazard.

Study No. 218 (Task Order UIC-24)

This study evaluated the toxic potential of WR6026 Dihydrochloride on reproductive capability in CD® male and female rats. WR6026 Dihydrochloride is being developed as an antileishmanial agent. Doses of 0, 3.0, 7.5, and 18 mg base/kg/day were selected on the basis of dose range-finding and developmental toxicity studies in female rats (UIC/TRL Study Nos. 170 and 171, respectively) and a three month toxicity study with a three month recovery period in male and female rats (UIC/TRL Study No. 091).

In the present study, doses of 0, 3.0, 7.5, and 18 mg base/kg/day were administered by daily gavage to male CD® rats for at least 54 days and to pregnant female CD® rats for 22 - 26 days in sperm-positive animals and for 33 days in sperm-negative females subsequently palpated pregnant. This included 29 days of dosing prior to cohabitation in males and 15 days of dosing prior to cohabitation in females. The results are summarized in Table 1.

One mid dose female was found dead on precohabitation day 13 in the absence of clinical signs and necropsy observations. No mortalities occurred in the high dose. Toxicity occurred in males at 18 mg base/kg/day and in females at 7.5 and 18 mg base/kg/day. Cyanosis seen as blue tongue was observed in both sexes at the high dose. Significantly reduced food consumption and body weights were noted in males at 18 mg base/kg/day essentially throughout the entire study. Decreased organ to brain weight ratios were noted in high and mid dose males for the epididymis. There were no treatment-related effects on sperm motility, count, or morphology in any WR6026 Dihydrochloride-treated group, and no apparent effect on the males' ability to impregnate the females. Females at 7.5 and 18 mg base/kg/day had significant reductions in food consumption and body weight gains during the precohabitation phase. Significantly reduced body weights continued during the gestation phase in females at the high dose. At 18 mg base/kg/day, insignificantly reduced numbers of *corpora lutea* occurred concomitant with significantly decreased numbers of implantations and viable fetuses. There were no effects on estrus cycle parameters in any group; mating and fertility were also unaffected by treatment with WR6026 Dihydrochloride at any dose level. This suggests that administration of 18 mg base/kg/day of

WR6026 Dihydrochloride for 15 days prior to mating affected oocyte maturation but not ovulation, mating behavior, implantation, or embryonic development. The no-observable-effect level (NOEL) for reproductive capability of males was 18 mg base/kg/day in spite of toxicity observed at this dose level. Based on the findings of altered maturation of oocytes at the high dose, the NOEL for females was 7.5 mg base/kg/day even though mating and fertility were unaffected at any dose level.

3. ADMINISTRATIVE AND LOGISTICAL MATTERS

No problems occurred.

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5. PERSONNEL

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